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Epigenetic signatures of childhood abuse and neglect: Implications for psychiatric vulnerability

SELF-ARCHIVING VERSION

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Abstract

Childhood maltreatment is a key risk factor for poor mental and physical health. Recently, variation in epigenetic processes, such as DNA methylation, has emerged as a potential pathway mediating this association; yet, the extent to which different forms of maltreatment may be characterized by unique vs shared epigenetic signatures is currently unknown. In this study, we quantified DNA methylation across the genome in buccal epithelial cell samples from a high-risk sample of inner-city youth (n = 124; age = 16-24; 53% female), 68% of whom reported experiencing at least one form of maltreatment while growing up. Our analyses aimed to identify methylomic variation associated with exposure to five major types of childhood maltreatment. We found that: (i) maltreatment types differ in the extent to which they associate with methylomic variation, with physical exposures showing the strongest associations; (ii) many of the identified loci are annotated to genes previously implicated in stress-related outcomes, including psychiatric and physical disorders (e.g. *GABBR1, GRIN2D, CACNA2D4, PSEN2)*; and (iii) based on gene ontology analyses, maltreatment types not only show *unique* methylation patterns enriched for specific biological processes (e.g. physical abuse and cardiovascular function), but also share a '*common'* epigenetic signature enriched for biological processes related to neural development and organismal growth. A stringent set of sensitivity analyses were also run to identify high-confidence associations. Together, findings lend novel insights into epigenetic signatures of childhood abuse and neglect, point to novel potential biomarkers for future investigation and support a molecular link between maltreatment and poor health outcomes. Nevertheless, it will be important in future to replicate findings, as the use of cross-sectional data and high rates of polyvictimization in our study make it difficult to fully disentangle the shared vs unique epigenetic signatures of maltreatment types. Furthermore, studies will be needed to test the role of potential moderators in the identified associations, including age of onset and chronicity of maltreatment exposure.

Keywords: DNA Methylation; epigenome-wide; child abuse; neglect; maltreatment; stress

Introduction

Childhood maltreatment, encompassing abuse and neglect, is a major public health concern that continues to affect up to one in four children worldwide, with often devastating developmental consequences (WHO, 2014). Children who experience maltreatment are at increased risk for a range of psychiatric problems, including anxiety, depression, post-traumatic stress, and antisocial behaviour (Cicchetti and Toth, 2005). The effects of maltreatment can extend well into adulthood, compromising relationship quality, economic productivity and physical health (Danese *et al.*, 2009).

The theory of latent vulnerability proposes that maltreatment exposure calibrates a range of biological and neurocognitive systems in line with a threatening and unpredictable early environment (McCrory and Viding, 2015). While potentially adaptive in the short term, such changes can increase vulnerability in the long term. Consistent with this view, numerous biological correlates of maltreatment have now been identified, including accelerated cellular ageing, neuroendocrine dysregulation, heightened inflammatory response as well as altered brain structure and function (Danese *et al.*, 2011, McCrory *et al.*, 2012, Shalev *et al.*, 2013). Recent evidence indicates that, as well as affecting common biological pathways, different forms of maltreatment may also exert unique effects. For example, while abuse has been associated with changes in neural circuitry underlying threat processing, neglect has been associated with biological adaptations to low-complexity environments (Sheridan and McLaughlin, 2014).

A key challenge for current research is to understand how, at a molecular level, these environmental exposures are translated into phenotypic variation. Epigenetic processes, such as DNA methylation (DNAm), which control the functional regulation of gene expression are of particular interest in this regard, as mounting evidence suggests they can be modified by environmental factors (Jaenisch and Bird, 2003). For example, animal studies have found that a number of environmental stressors, such as poor maternal care, induce stable alterations in DNAm in the regulatory regions of several HPA axis genes (e.g. the glucocorticoid receptor), which in turn influence responses to future stressors (Turecki and Meaney, 2014). Similarly, a small number of human studies have documented a link between childhood maltreatment and aberrant DNAm in genes important for stress-response, immune function and neurodevelopment (Lutz and Turecki, 2014). DNAm has also been shown to regulate a wide range of neurobiological processes, including neurogenesis, synaptic plasticity, learning and memory (Baker-Andresen *et al.*, 2013, Day *et al.*, 2013) and aberrations in DNAm have been observed in a range of diseased states, including stress-related psychiatric disorders such as post-traumatic stress and major depression (Bergman and Cedar, 2013, Klengel *et al.*, 2014).

To date, most epigenetic studies of maltreatment have focused on variation in the vicinity of a limited set of pre-selected candidate genes (i.e. *GR*, *FKBP5*, *BDNF and 5-HTT*) (Lutz and Turecki, 2014). As such, little is known about the broader effect of maltreatment on DNAm across the genome. This is a substantial limitation in light of the fact that maltreatment impacts multiple aspects of functioning, across psychological, physical, and social domains. Furthermore, existing studies have primarily examined global maltreatment (Labonte *et al.*, 2012, Prados *et al.*, 2015, Suderman *et al.*, 2014, Yang *et al.*, 2013), so that the extent to which different maltreatment types may have common vs distinct epigenetic signatures is unclear. To address these outstanding questions, we explored the relationship

between DNAm and five types of maltreatment in a sample of high-risk youth, using genome-wide DNAm data drawn from buccal epithelial cells.

Methods and Materials

Participants

The current sample was recruited as part of a larger study examining the effects of developmental adversity on individual functioning (n = 204, age range = 16-24 years). Analyses only included participants for whom DNAm data was available (n = 124). Youth from deprived inner London areas were recruited through multiple channels including inner-city colleges, internet websites and a charity providing services and support to self-referred youth. The sample was 53% female and ethnically diverse (49% White, 33% Black, 18% other). The study was carried out in accordance with the latest version of the Declaration of Helsinki. The study design was reviewed and approved by the UCL Research Ethics Committee (*ID No*: 2462/001) and all participants provided informed consent prior to participation, after the nature of the procedures had been fully explained. Further details of the sample and recruitment procedures are available elsewhere (Cecil *et al.*, 2014).

Measures

Childhood maltreatment – Childhood maltreatment was assessed using the 28-item, self-report Childhood Trauma Questionnaire (CTQ; Bernstein and Fink, 1998). The CTQ screens for experiences of maltreatment "while growing up" and comprises of 5 continuous subscales: emotional abuse, sexual abuse, physical abuse, emotional neglect and physical neglect. The scales show high internal consistency in our sample (α = .70 – .97). For descriptive purposes only, we also classified participants as having experienced maltreatment (i.e. yes/no) if they scored above the 'Low' threshold specified by the CTQ manual for at least one maltreatment type. By including 'I currently feel unsafe at home' as an additional yes/no item we were able to ascertain that none of the participants in the study were currently vulnerable to violence in the domestic environment (e.g. by family or partner). As such, the present study investigates the effects of childhood (i.e. past) maltreatment.

DNA methylation – DNA was extracted from buccal epithelial cells using procedures described in Freeman *et al.* (2003). 500ng of high molecular weight DNA was subjected to sodium bisulfite conversion using the EZ-DNA methylation kit (Zymo Research, Orange, CA, USA) using the manufacturers standard protocol. DNAm was quantified using the Illumina HumanMethylation450 BeadChip (Illumina, USA) with arrays scanned using an Illumina iScan (software version 3.3.28). The Illumina 450K array interrogates >485,000 probes covering 99% of Reference Sequence (RefSeq) genes, with an average of 17 CpG sites per gene region. As the samples were run in a single batch, there was no need for batch correction. To account for potential chip and position effects, we randomized sample chip allocation and placement on the chip. Initial data quality control was conducted using GenomeStudio (version 2011.1) to determine the status of staining, extension, hybridization, target removal, bisulfite conversion, specificity, non-polymorphic and negative controls. Samples that survived this stage were checked for concordance between their reported and assessed sex and then quantile normalised using the *dasen* function within the *wateRmelon*

package (wateRmelon_1.0.3; Pidsley *et al.*, 2013) in R. Probes were removed if they were cross-reactive, polymorphic, used for sample identification on the array, had a SNP at the single base extension with a minor allele frequency larger than 5% (i.e. common polymorphisms) or were located on the Y chromosome, leaving a total of 413,239 probes (Chen *et al.*, 2013; Price *et al.*, 2013). DNAm levels are indexed by beta values (ratio of methylated signal divided by the sum of the methylated and unmethylated signal, M/M+U).

Data Analysis

All analyses were performed within the R statistical environment (version 3.0.1). Methylation data was regressed for sex, age and self-reported ethnicity to account for potential confounding effects (Liang and Cookson, 2014). The analysis proceeded in three steps. First, we ran five independent epigenome-wide association analyses – one for each maltreatment type measured – using linear regression models. Probes were considered significant if they survived a False Discovery Rate (FDR) correction of $q < 0.05$. Only maltreatment types that were associated with at least one FDR-corrected probe were carried forward to the next step. Second, we identified which probes were most consistently associated with *all* types of maltreatment, by ranking them in order of average standardized effect size. Third, we examined enriched biological pathways for genes that were associated only with one type of maltreatment vs those that were associated with all maltreatment types, using an optimized gene ontology method that controls for a range of potential confounds, including background probe distribution and gene size (see **OS1** for details). More specifically, genes were considered 'unique' if the probes annotated to them were specifically associated with one maltreatment type and none other (i.e. $p<0.005$ with one type of maltreatment, and $p>0.05$ with the other two types of maltreatment). Conversely, genes were considered 'shared' if probes annotated to them were associated across maltreatment types ($p<0.05$ consistently across all three forms of maltreatment). To calculate statistical power to detect effects, we used the pwr package with a p-value threshold of 1.00E-06 to reach 80% power (Tsai & Bell, 2015) in the context of a linear regression model. Results showed that, with a sample size of 124 individuals, we could expect to detect an effect size of ≥ 0.32, indicating that the study is appropriately powered for detecting medium-to-large effects.

Results

Descriptives and correlations between study variables shown in **Table 1**. Based on the CTQ cut-off, the majority of youth in the current study reported having experienced at least one form of maltreatment while growing up (68%; $n = 84$). The reported experiences were often severe: 75% of physically abused youth were left with bruises or marks as a result of injuries; 50% of sexually abused youth reported being threatened; and 60% of physically neglected youth reported not having enough to eat. Poly-victimization was common, with 74% of maltreated youth reporting two or more forms of maltreatment, consistent with previous studies (Radford *et al.*, 2011).

********** **Table 1**

Maltreatment types differ in the extent to which they associate with DNAm variation
We first examined associations between DNAm across the genome and individual maltreatment types (measured continuously). No probes were identified as being differentially methylated at a FDR<0.05 for emotional abuse and emotional neglect (**OS2**); as such, these two types of maltreatment were excluded from further analysis. With regards to other maltreatment types, the number of differentially methylated probes (DMPs) was: 34 for physical abuse, 7 for sexual abuse, and 118 for physical neglect. The 10 top-ranked DMPs for each of these maltreatment types are displayed in **Table 2** (see **OS3** for a complete table of FDR-corrected probes). Associations between DNAm and each maltreatment type are graphically represented in **Figure 1A** (see **OS4** for quantile-quantile plots for each analysis).

Physical abuse: The top-ranked DMP, cg20000641, was significantly hypermethylated with increased exposure to physical abuse (*p*=3.54E-11, q=1.47E-05; **Table 2A**, **Figure 1B**). This probe is located in the promoter region of *PSEN2*, a gene encoding a presenilin enzyme involved in amyloid precursor protein processing that is robustly implicated in Alzheimer's disease (O'Brien and Wong, 2011). Two top-ranked probes were annotated to *SMC1A* (cg02353937: *p* = 2.90E-09, q=7.89E-04; cg22311608: *p* = 8.45E-08, q=0.01), a gene involved in chromosomal maintenance and DNA repair (Kim *et al.*, 2002). Also of interest were DMPs annotated to genes highly expressed in the brain, including *SHC2* (cg19736040: *p* = 1.38E-06, q=0.05), involved in neurotrophin-activated Trk receptor signaling within cortical neurons and synaptic plasticity in the hippocampus (Epa *et al.*, 2004), and *IMPACT* (cg03013329: *p* = 2.10E-06, q=0.05), encoding a protein that facilitates neurite outgrowth and modulates kinase activation in neurons (Roffe *et al.*, 2013).

Sexual abuse: The top-ranked DMP associated with sexual abuse (cg17106653, *p*=4.16E-09, q=1.72E-03; **Table 2B**, **Figure 1B)** was located in the promoter region of the glutamate receptor *GRIN2D*, a gene implicated in CNS plasticity and excitatory synaptic transmission (Traynelis *et al.*, 2010). Other annotated genes of interest include *MGMT* (cg26528551: *p* = 6.84E-08, q=0.01) implicated in DNA repair mechanisms and *DIP2C* (cg23983710: *p* = 5.83E-07, q=0.03), a gene primarily expressed in the brain but whose biological function is poorly understood.

Physical Neglect: The top-ranked DMP associated with physical neglect was cg00691266 in *EVPN* (*p*=1.01E-07, q=0.01; **Table 2C**), encoding a protein involved in epidermal growth. Several probes were annotated to genes related to brain function, including *SYNJ2* (cg27083825: *p* = 1.02E-07, q=0.01; **Figure 1B**), involved in nervous system development and neuronal vesicle uncoating (Montesinos *et al.*, 2005); and *GABBR1* (cg18116160: *p* = 4.01E-07, q = 0.02) a GABA class B receptor important for inhibitory synaptic transmission (Kumar *et al.*, 2013). Probes annotated to genes involved in histone regulation were also identified, including *SETDB1* (cg17918089: *p* = 1.14E-07, q=0.01), *JADE1* (cg09863040: *p* = 2.10E-07, q=0.01), and *HIST1H1A* (cg08054907: *p* = 5.12E-07, q=0.02).

Sensitivity analyses: Given that quantile-quantile plots from our epigenome-wide analyses indicated skewing of significant results (**OS4**), we carried out **a set of** sensitivity analyses to test the robustness of the identified associations. **First**, to minimize the influence of unknown

confounders, we ran a surrogate variable analysis (sva package in R; Leek et al., 2012) that enabled us to identify unwanted sources of variation in our DNA methylation data. The analysis identified 11 surrogate variables representing different sources of variation/noise. We then re-ran associations between each form of maltreatment and DNAm additionally controlling for these 11 surrogate variables. While variations in significance levels were observed across a number of probes (both increases and decreases in p-value), all sites remained significantly associated with maltreatment severity **($p < 0.05$)**, with the exception of two probes related to physical neglect (see **OS5**). A total of 45 sites (physical abuse: $n = 26$; sexual abuse: $n = 7$ and physical neglect: $n = 12$) also survived genome-wide correction ($q < 0.05$). Overall, associations with physical and sexual abuse were the least affected by unmeasured confounding, and those with physical neglect were the most affected.

Second, to examine the potential influence of outliers, we applied the winsorize function within the robustHD package to the DNAm data and reran epigenome-wide analyses, controlling for covariates. The winsorizing method uses censoring rather than exclusion, which is preferable with small sample sizes (Sheskin, 2003). Specifically, for each probe, values <5% or >95% of the score distribution were transformed to match the closest value within this percentile, which enables scores to maintain their relative weight without exerting an undue influence on the linear regression model. Although the vast majority of associations remained significant after winsorizing (97%; $p<0.05$), none of the transformed DMPs survived genome-wide correction ($q>0.05$; **OS5**). It is important to note, however, that this sensitivity analysis is highly conservative as it transforms, for each probe, scores at the tail ends of the distribution regardless of presence or absence of outliers. As such, this approach reduces the range of methylation scores across all probes, resulting in more limited variability.

Third, we performed bootstrapping as a complement to winsorizing using Mplus (version 6.1.1; Muthen & Muthen, 2011). Bootstrapping is advantageous with small samples as it derives an approximation of the sampling distribution via repeated resampling of the available data to yield bias-corrected 95% confidence intervals (CI). Associations were considered significant if bootstrapped 95% CIs (10,000 times) did not cross zero. The number of DMPs that survived bootstrapping were: $n = 29$ for physical abuse, $n = 1$ for sexual abuse, and $n = 108$ for physical neglect. Hence, sites associated with sexual abuse were the most affected by this sensitivity analysis, potentially reflecting the low number of individuals scoring high on this exposure, resulting in more extreme values.

Based on the above results, we compiled a list of high-confidence associations (see **OS5**), in order to highlight DMPs that were the most robustly implicated across the three sensitivity analyses and as such may be particularly promising candidates for prioritization in future studies. High-confidence associations were defined as having (i) an SVA q-value < 0.05, (ii) a winsorized p-value < 0.05, and (iii) significant bootstrapped 95% CIs. The resulting number of high-confidence associations for each exposure were: $n = 20$ for physical abuse, $n = 1$ for sexual abuse and $n = 10$ for physical neglect.

********** **Table 2**********

********** **Figure 1**********

Variability in certain DNAm loci consistently associate with all maltreatment types
The top 20 hyper- and hypomethylated DMPs consistently associated with all three maltreatment types (i.e. physical abuse, sexual abuse and neglect) are shown in **Table 3**, ranked by average standardized effect size. The top-ranked hypermethylated probe, cg08796898 (**Figure 1C**), is located in *HUWE1,* an important regulator of neural proliferation linked to intellectual disability (Vandewalle *et al.*, 2013). Other genes annotated to hypermethylated probes include: (i) *CACNA2D4* (cg27516159), identified as a shared risk locus for multiple psychiatric disorders (Smollen *et al.*, 2013); (ii) *WBSCR17* (cg25579180), a gene widely expressed in the brain and associated with neurodevelopmental delay (Nakamura *et al.*, 2005); (iii) *LRP4* (cg12627354), involved in neuromuscular junction maintenance and acetylcholine signaling; and (iv) *GRB10* (cg26163537), an insulin modulator.

With regard to hypomethylated probes, we identified multiple markers annotated to genes that are involved in response to environmental stimuli, including: (i) two probes in *RPTOR* (cg07870603, cg09596252), a gene involved in cell growth regulation in response to climatic factors, nutrient and insulin levels (Sun *et al.*, 2010); and (ii) *GJD3* (cg22896075), which encodes a connexin thought to facilitate environmental adaptation by regulating physiological processes such as neuronal excitability (Belousov and Fontes, 2013). Another gene, *DNAJB6* (cg27496299; **Figure 1C**), has been found to reduce cellular toxicity and act as a molecular chaperone for neuronal proteins, including huntingtin (Mansson *et al.*, 2014). Finally, *TIAM2* (cg01786585) has been implicated in neurogenesis, particularly in the hippocampus, as well as promoting neural migration in the cerebral cortex (Chiu *et al.*, 1999).

DNAm variation implicates both maltreatment-specific and shared functional pathways
As a final step, we examined enriched biological pathways for genes that were either (i) annotated to DMPs that associated with only *one* type of maltreatment ($p<0.005$); or (ii) annotated to DMPs that associated with *all* maltreatment types ($p<0.05$ across physical abuse, sexual abuse and neglect). Following these criteria, neglect showed 'unique' epigenetic variation in probes annotated to a larger number of genes (n =329) than physical (n =119) and sexual abuse (n = 47). A considerable proportion of genes showed epigenetic variation across all three maltreatment type (i.e. 'shared' genes; n = 2,348; consistent direction of effects).

Gene ontology analyses were performed for the above sets of genes (except sexual abuse, due to the low number of annotated genes), controlling for a range of potential confounds, including background probe distribution and gene size (**Figure 2**; see **OS1** for details). Results indicated that genes associated with DNAm variation specific to *physical abuse* were enriched for biological processes including cardiovascular function (e.g. cardiac muscle hypertrophy, heart rate), fear response, and wound healing (1.04E-12 <p<7.65E-03), while those associated with *neglect*-specific variation were enriched for regulation of cholesterol efflux, as well as processes related to cellular function and metabolism (2.91E-11 <p<1.04E-02; see **OS6** for a full list of terms). In contrast, genes associated with epigenetic variation *shared* across maltreatment types were significantly enriched for biological processes primarily related to regulation of nervous system development (e.g. neurogenesis, glial proliferation, neurotransmitter biosynthesis, oligodendrocyte development) and

organismal growth (e.g. organ morphogenesis, negative regulation of growth, response to growth factor; see **OS7** for a full list of terms; 4.20E-10<*p*< 1.44E-02).
********** **Figure 2**********

Discussion

This study is the first to characterize genome-wide DNAm patterns associated with different types of childhood abuse and neglect. Strengths include the availability of quantitative data on a range of different exposures, the inclusion of a sample of youth featuring high rates of adversity, and the analysis of methylome-wide data. Here, we highlight three key findings: (i) specific types of maltreatment, particularly physical exposures, are associated with DNAm variation at multiple loci; (ii) many of the identified loci are annotated to genes previously implicated in psychiatric and medical disorders; and (iii) gene ontology analyses indicate that, while maltreatment types show distinct patterns of methylomic variation, they also share a common epigenetic 'signature' enriched for biological processes related to neural development and organismal growth. The use of stringent sensitivity analyses further enabled us to identify high-confidence associations, which point to promising candidate loci for prioritization in future studies.

Findings lend novel insights into the relationship between maltreatment and DNA methylation

Of the maltreatment types investigated, we found that physical exposures – including physical abuse, sexual abuse and physical neglect – all associated with epigenetic variation at multiple loci, with neglect showing the largest number of DMPs after genome-wide correction (despite having comparable prevalence rates to physical abuse). In contrast, no significant loci were identified for emotional abuse and emotional neglect (after multiple correction). This was unexpected given that emotional abuse, in particular, has been recognized as an important independent predictor of poor individual functioning (Rees, 2010). Consequently, more work is required to clarify whether results reflect a true lack of associations or whether other factors may be at play (e.g. challenges with the operationalization of emotional abuse; polyepigenetic effects of smaller magnitude; tissue specificity). It is also interesting to note that we identified an unexpectedly high number of associations between maltreatment types and DNAm sites located on the X-chromosome. Given that sex was controlled for in the analyses, these results suggest that maltreatment exposure may influence these DNAm sites similarly for boys and girls. Although sex differences were not examined in the present study due to sample size limitations, it will be of interest in future to test the potential role of sex in the relationship between maltreatment and DNAm.

Comparability with previous findings on maltreatment and DNAm is limited by the fact that, while other epigenetic studies have examined maltreatment as a global construct, we investigated DNA methylation profiles associated with specific forms of abuse and neglect. As such, a strict test of replication was not possible. Furthermore, past studies have varied widely on factors such as choice of tissue (e.g. saliva, blood or brain), DNAm platform (e.g.

Illumina 450k vs MeDIP), methodology (e.g. filtering of probes, covariates), maltreatment measure (e.g. self-report vs official records), population (e.g. community vs clinical) and sample characteristics (e.g. age, sex), making it difficult to assess our findings in the context of previous work in the area.

Nevertheless, we note here two of the most similar studies to ours, which have also used the Illumina 450k platform in peripheral tissues to investigate methylome-wide associations with childhood maltreatment. The first study (Yang *et al*. 2013) used DNA from saliva specimens to compare methylomic differences between a sample of maltreated and non-maltreated children, using a case-control design. The second study (Prados *et al*. 2015) used blood samples to compare DNAm patterns in adults with borderline personality disorder and exposure to high levels of childhood maltreatment vs adults with major depression disorder who had experienced low levels of childhood maltreatment (assessed using the same measure as our study, the CTQ). Comparing findings from our respective genome-wide analyses, we identified one overlapping DNAm site that was associated with maltreatment (after genome-wide correction) across both our study and Yang *et al*.'s (*CILP2*$_{cg01487433}$), and no overlap with the DNAm sites reported in Prados *et al*'s study. In addition, we identified several overlapping genes between studies (29 with Yang's *et al*.'s study; 1 with Prados *et al*.'s study) that had at least one genome-wide significant probe annotated to them. However, because these genes varied widely in DNAm probe coverage, it is unclear whether they were identified across studies because they robustly associate with maltreatment or because they may have had a larger number of DNAm probes annotated to them. As a whole, DMPs were found to differ between our study and previous ones. As such, it will be important in future to establish whether discrepancies may reflect the examination of global vs individual forms of maltreatment, methodological differences between studies or the presence of false positives, all issues that should be considered when interpreting the present findings.

We note that the use of sensitivity analyses in the present study (surrogate variable analysis, winsorizing and bootstrapping) enabled us to identify a subset of DMPs that were most robustly associated with maltreatment exposure (i.e. 'high-confidence associations'; n = 31, 20% of total DMP set), and consequently may show promise as candidate loci for further investigation. Of interest, DMPs associated with physical neglect were most affected by the surrogate variable analysis, suggesting that the methylomic signature of this maltreatment type may in part reflect other sources of variability (e.g. genetic architecture, unmeasured exposures, confounding sources). In contrast, DMPs associated with sexual abuse were the most affected by the winsorizing and bootstrapping analyses, which may instead reflect the low number of individuals reporting high levels of this exposure. In general, associations between DNAm and physical abuse were the most robust, with 59% of DMPs (n = 20) classified as high-confidence. While these findings may be used in future to inform locus-prioritization, it is important to note that the identification of high-confidence associations was based on a stringent set of criteria, so that the extent to which 'lower-confidence' DMPs may be robustly associated with maltreatment exposure will still need to be established via replication in independent samples.

The identified DNA methylation markers support a molecular link between maltreatment and poor health outcomes

Our analyses identified multiple DMPs annotated to genes previously associated with psychiatric and medical disorders. The top-ranked DMP for physical abuse was located in the promoter region of *PSEN2*, a gene implicated in neurodegeneration and Alzheimer's disease (O'Brien and Wong, 2011). Although we are not aware of any study directly investigating the link between physical abuse and these conditions in advanced age, early life factors, including stress and the quality of the maternal relationship, have been associated with both neuropsychological impairment and dementia (Pechtel and Pizzagalli, 2011, Vaillant *et al.*, 2014). It will therefore be of interest to test whether *PSEN2* methylation mediates the effect of abuse on cognitive function and neurodegenerative risk. Maltreatment-associated DNAm changes were additionally identified in a number of other genes implicated in cognitive deficit and intellectual disability, including *HUWE1, WBSCR17* and *SMC1A* (Deardorff *et al.*, 2007, Nakamura *et al.*, 2005, Vandewalle *et al.*, 2013).

Findings also suggest a role of other loci previously implicated in psychopathology. Notably, one of the most hypermethylated markers across maltreatment types was located in *CACNA2D4*. Genetic variation within this gene has been identified as a *shared* risk locus for multiple psychiatric conditions that are associated with experience of childhood adversity, including attention deficit-hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia (Smollen *et al.*, 2013). Interestingly, expression levels of this gene have been found to modulate neural hippocampal activity during emotional processing as well as prefrontal activity during executive tasks (Bigos *et al.*, 2010) – functional patterns that are disrupted in maltreated individuals (Hart and Rubia, 2012). Furthermore, genetic variation in *SYNJ2*, another gene identified in our epigenome-wide analyses, has been linked to corpus callosum abnormalities (Edwards *et al.*, 2014), which are robustly associated with maltreatment (McCrory *et al.*, 2012). Other psychopathology-relevant genes included glutamate and GABA receptors (*GRIND2D, GABBR1*), which play a key role in excitatory and inhibitory neurotransmission, respectively (Kumar *et al.*, 2013, Yamamoto *et al.*, 2015). Prospective studies will be needed to explicitly test whether the DNAm sites identified in the present study associate with psychopathological outcomes and, if so, whether they may mediate the influence of abuse and neglect on later mental health.

Gene ontology analyses contribute to a better understanding of biological pathways that may be affected by childhood abuse and neglect.

Gene ontology analyses were performed to explore biological pathways that may be uniquely affected by specific forms of maltreatment vs those 'shared' across maltreatment types. With regards to 'maltreatment-specific' pathways, we found that physical abuse was primarily associated with DNAm variation in the vicinity of genes enriched for cardiovascular processes, including regulation of heart rate and myocardial hypertrophy. This is consistent with evidence from epidemiological studies of elevated risk of heart disease among individuals who were physically abused as children, even after controlling for other childhood stressors, adult lifestyle factors and unhealthy behaviors (Fuller-Thomson *et al.*,

2010, Springer *et al.*, 2007). Other enriched processes of interest related to wound healing, fear response and regulation of stress-activated protein kinase signaling. These pathways are in line with growing evidence from neuroendocrine and imaging studies of an association between physical abuse, HPA axis dysregulation and altered threat processing at neural and behavioral levels (Carpenter *et al.*, 2011, McCrory *et al.*, 2011, Turecki and Meaney, 2014). In contrast, the most enriched biological process specifically linked to neglect was regulation of cholesterol efflux, followed by other processes including DNA damage response, ribosomal function and zymogen activation. Although this finding necessitates replication, it is worth noting that these processes have been shown to respond to diet and nutrient availability (Fenech and Bonassi, 2011, Mizushima *et al.*, 2004), which may be particularly compromised in youth who have experienced neglect (as evidenced in our sample). Together, our data suggest that physical abuse and neglect may affect epigenetic regulation of separate biological pathways, which may in part underlie differential effects observed at a phenotypic level (Sheridan and McLaughlin, 2014). Sexual abuse could not be examined due to the limited number of genes showing 'unique' epigenetic variation, which may reflect lack of power due to lower prevalence rates in our sample.

In addition to showing specific epigenetic variation, maltreatment types were also found to share a common methylomic signature, primarily enriched for processes related to neurodevelopment and organismal growth. This is consistent with a large body of evidence from animal and human studies documenting the impact of maltreatment and early life stress on brain structure, function and development. For example, maltreatment has been consistently associated with reduced volume of the corpus callosum, prefrontal cortex and hippocampus (Hart and Rubia, 2012, McCrory *et al.*, 2012). Broader developmental delay and growth failure is also well established amongst maltreated children (Leslie *et al.*, 2005, Olivan, 2003). Findings are also in line with previous epigenetic studies that have documented an association between global maltreatment exposure and DNAm changes in genes enriched for neural (e.g. Labonte *et al*, 2012: *'neuron projection', 'dendrite'*; Yang *et al*, 2013: *'neurogenesis', 'axonal guidance'*) and developmental (e.g. Suderman et al, 2014: *'multicellular organismal development'*) processes. In future, it will be important to explore whether epigenetic regulation of these 'core' processes in response to maltreatment is functionally relevant at a transcriptomic level, and whether they relate directly to other biological markers of neurodevelopment and growth, such as imaging data and markers of cellular ageing.

Limitations and future directions

The current findings should be interpreted in light of a number of important limitations. First, although rates of maltreatment in our sample were high, analyses were based on a modestly sized group of inner-city youth, which precluded the possibility of addressing more nuanced research questions (e.g. sex differences). Furthermore, our assessment of maltreatment was based on self-reports that lacked information regarding maltreatment timing and duration, both of which are likely to moderate the association between maltreatment and DNA methylation. In future, it will be important to replicate findings using larger samples, ideally

featuring externally-validated maltreatment histories that will make it possible to investigate how DNAm patterns may vary by age of onset and chronicity of exposure to different forms of maltreatment. Second, due to the high rates of polyvictimization in our sample, it was not feasible to compare methylomic patterns between individuals who experienced single forms of maltreatment in isolation. Instead, we examined methylome-wide associations for each maltreatment type dimensionally, and then explored the presence of unique vs shared signatures at a functional pathway level, as opposed to a DNAm site level. Even though associations were always strongest for the maltreatment type under investigation, it is therefore still possible that the identified sites may have in part reflected the combined effect of exposure to multiple maltreatment types. Furthermore, the identification of unique vs shared biological pathways linked to maltreatment were based on gene ontology analyses, which can be susceptible to bias (Timmons *et al.*, 2015), and consequently will necessitate replication. Third, data on smoking status and medication use were not available in the present study. As these exposures have been shown to alter DNAm patterns (particularly in blood; Gao *et al*., 2015), it will be important to replicate findings controlling for these potential confounders. It is noteworthy, however, that none of the DNAm sites identified in the present study overlapped with those found to be robustly affected by smoking in a recent systematic review (i.e. DNAm sites associated with smoking in at least three independent reports; Gao et al., 2015). Fourth, findings were based on DNAm from buccal cell samples and can thus only be considered to represent biomarkers of exposure. Although buccal epithelial cells have been shown to converge more strongly with brain methylation patterns compared to other peripheral tissues (e.g. blood; Smith *et al.*, 2015), further investigation will be needed to establish the relevance of our findings to the brain. Because we did not have access to RNA samples in the present study, we were also unable to establish the extent to which the identified DNAm sites associate with gene expression levels. As such, the analysis of transcriptomic data will be important for assessing the functional significance of the observed DNAm changes. Finally, the cross-sectional nature of the study meant that we were unable to establish the causal role of the identified DMPs. Longitudinal assessments featuring repeated DNAm measures will be needed to explore prospective interrelations between maltreatment exposure, DNAm and developmental outcomes.

Conclusions

The current findings shed new light on how maltreatment may alter epigenetic mechanisms that regulate gene expression, providing a possible biological link between early adversity and poor health outcomes. Different forms of maltreatment were found to have distinct as well as shared signatures, pointing to a complex relationship between the nature of early adverse experience and multiple biological processes relevant for healthy normal development.

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Figure legends:

Figure 1. Associations between DNA methylation and maltreatment types.
(A) Manhattan plots for physical abuse, sexual abuse and physical neglect; **(B**) scatterplots of DMPs indicated in **(A**) for each maltreatment type; and **(C)** scatterplots of hyper- and hypo-methylated DMPs *across* maltreatment types. *N.b.* The dotted line represents FDR correction (i.e. DMPs above the line are significant at $q<0.05$).

Figure 2. Unique vs shared enriched biological processes across maltreatment types.
Significantly enriched biological processes for genes uniquely associated with each maltreatment type (physical abuse [green], physical neglect [blue]) vs those shared across maltreatment types (red), based on GO analysis. GO analysis was not run independently for sexual abuse (SA) due to limited gene *n*. Circles represent GO terms that survive FDR correction. The X axis represents -log(10) p values. The opacity of the circles indicates level of significance (darker = more significant). The size of the circles indicates the percentage of genes in our results for a given pathway compared to the total number of genes in the same pathway (i.e. larger size = larger %; range = 6.51% - 100%). *Abbreviations*: PA, physical abuse; PN, physical neglect; SA, sexual abuse.

Table 1. Descriptives and correlations between study variables.

Maltreatment type	M *(SD)*	% above threshold *(n)*	1	2	3	4	Sex	Age	Ethnicity[b]			
									White	Black	Mixed	Asian
1. Emotional abuse	10.06 *(4.99)*	50.8 *(63)*	–				.01	.17	-.13	.19*	-.07	.00
2. Physical abuse	7.82 *(4.70)*	33.1 *(41)*	.57***	–			.01	.09	-.16	.26**	-.08	-.06
3. Sexual abuse	6.14 *(3.51)*	17.0 *(21)*	.41***	.25***	–		.05	.13	-.11	.12	-.01	-.04
4. Emotional neglect	10.47 *(4.81)*	49.2 *(61)*	.73***	.55***	.35***	–	-.03	.18*	-.20*	.21*	-.09	.12
5. Physical neglect	7.55 *(3.61)*	32.3 *(40)*	.65***	.60***	.36***	.71***	-.07	.14	-.22*	.31***	-.11	.00

N.B. Intercorrelations between maltreatment types, and their association with socio-demographic characteristics (age, sex and ethnicity). A product-moment correlation is used for associations between two continuous variables, while a point-biserial correlation is used for associations between one continuous and one dichotomous variable (i.e. sex, ethnicity). Bivariate correlations significant at: *p <.05, **p < .01, ***p < .001.

[a] For descriptive purposes, this column shows the percentage of participants who scored above the 'Low' maltreatment threshold specified by the CTQ manual.

[b] Each ethnic group coded as 1 = yes and 0 = no, based on self-reported ethnicity.

Table 2. Top 10 DMPs associated with physical abuse, sexual abuse and physical neglect.

CpG probe	*Gene*	Chr	Genomic location	Position	*B diff.*[a]	*Std .B*[b]	P-value	FDR (q)
A. Physical abuse (*N* FDR-corrected DMPs = 34)								
cg20000641	*PSEN2*	1	TSS1500	227058046	0.14	0.55	2.53E-11	1.47E-05
cg02353937	***SMC1A/RIBC1***	X	TSS1500	53449152	0.09	0.50	2.90E-09	7.89E-04
cg15440363	*GPD1L*	3	TSS200	32148023	0.05	0.48	2.06E-08	3.64E-03
cg04412054	*GALNS*	16	Body	88897539	-0.12	-0.46	5.69E-08	0.01
cg22311608	***SMC1A/RIBC1***	X	TSS200	53449829	0.09	0.46	8.45E-08	0.01
cg24365098	*C11orf84*	11	3'UTR	63594804	-0.12	-0.45	1.43E-07	0.01
cg03561071	*[FAT3]*	11	--	91844355	-0.11	-0.44	4.28E-07	0.03
cg26454299	*PPP3CA*	4	--	102268957	0.10	0.43	5.58E-07	0.03
cg25047485	*[ARHGAP39]*	8	--	145848944	-0.11	-0.43	8.14E-07	0.04
cg04524770	*LMF1*	16	Body	946408	-0.11	-0.42	9.96E-07	0.04
B. Sexual abuse (*N* FDR-corrected DMPs = 7)								
cg17106653	*GRIN2D*	19	TSS1500	48897279	0.22	0.50	4.16E-09	1.72E-03
cg00974464	*PRDM15*	21	Body	43254115	-0.12	-0.46	6.02E-08	0.01
cg26528551	*MGMT*	10	Body	131445415	-0.09	-0.46	6.84E-08	0.01
cg10795666	*MLNR*	13	1stExon	49794635	0.09	0.45	2.23E-07	0.02
cg02618355	*MYOM2*	8	Body	2024368	-0.09	-0.44	2.88E-07	0.02
cg26513050	*DIAPH2/RPA4*	X	Body	96138983	-0.18	-0.44	2.91E-07	0.02
cg23983710	*DIP2C*	10	Body	370756	-0.16	-0.43	5.83E-07	0.03
C. Physical neglect (*N* FDR-corrected DMPs = 118)								
cg00691266	*EVPL*	17	Body	74015089	-0.08	-0.46	1.01E-07	0.01
cg27083825	*SYNJ2*	6	Body	158453594	-0.07	-0.45	1.02E-07	0.01
cg17918089	*SETDB1*	1	Body	150899251	0.08	0.45	1.14E-07	0.01
cg10094509	*MAP2K4P1/CHIC1*	X	TSS1500	72783579	0.09	0.45	1.16E-07	0.01
cg09863040	*JADE1*	4	TSS200	129730729	0.02	0.44	2.10E-07	0.01
cg08796898	*HUWE1*	X	5'UTR	53713664	0.14	0.44	3.59E-07	0.02

cg20668974	*ZNF827*	4	Body	146857981	0.06	0.44	3.63E-07	0.02
cg18116160	*GABBR1*	6	Body	29575145	-0.04	-0.44	4.01E-07	0.02
cg09084892	*FAT1*	4	Body	187557837	-0.12	-0.43	4.35E-07	0.02
cg08054907	*HIST1H1A*	6	TSS1500	26019358	-0.09	-0.43	5.12E-07	0.02

N.B. Gene names in brackets indicate the most proximal genes to the CpG probe based on Genome Studio. Genes with multiple significant probes are highlighted in blue.

[a] Beta differences indicate the overall difference (% methylation change) between the predicted unstandardized minimum and maximum values of the linear model.

[b] Standardized Beta estimates are used as a measure of effect size, where an effect of 0.10 is small effect, an effect of 0.24 is a medium effect, and an effect of 0.37 is a large effect.

Table 3. Top 20 hyper- and hypo-methylated DMPs across maltreatment types (physical abuse, sexual abuse and neglect), ranked by average effect size.

CpG probe	Gene	Chr	Genomic location	Position	*Physical Abuse*		*Sexual Abuse*		*Neglect*		*Average StdB*
					StdB	*p*-value	*StdB*	*p*-value	*StdB*	*p*-value	
A. Hypermethylated											
cg08796898	*HUWE1*	X	5'UTR	53713664	0.38	1.10E-05	0.21	2.19E-02	0.44	3.83E-07	0.34
cg21239691	*GEMIN8*	X	TSS200	14048191	0.42	1.24E-06	0.18	4.38E-02	0.39	7.20E-06	0.33
cg04704856	*FMOD*	1	1stExon	203320190	0.32	2.46E-04	0.27	2.61E-03	0.37	2.36E-05	0.32
cg12986338	*[PDE1C]*	7	--	32338950	0.28	1.39E-03	0.31	4.15E-04	0.35	6.27E-05	0.32
cg23436576	*TDH*	8	Body	11204132	0.35	7.16E-05	0.23	8.75E-03	0.35	6.17E-05	0.31
cg25051341	*PRDM13*	6	Body	100061307	0.27	2.79E-03	0.28	1.40E-03	0.38	1.30E-05	0.31
cg19637330	*[PAX7]*	1	--	19110922	0.26	3.73E-03	0.31	4.51E-04	0.36	3.98E-05	0.31
cg26163537	*GRB10*	7	TSS1500	50861592	0.36	3.84E-05	0.19	3.76E-02	0.38	1.37E-05	0.31
cg25579180	*WBSCR17*	7	Body	71098623	0.36	4.53E-05	0.32	3.03E-04	0.25	5.24E-03	0.31
cg12627354	*LRP4*	11	TSS1500	46940434	0.26	3.29E-03	0.28	1.56E-03	0.38	1.67E-05	0.31
cg08121755	*[KRT80]*	12	--	52545978	0.27	2.15E-03	0.34	1.39E-04	0.29	9.09E-04	0.30
cg03309770	*TVP23A*	16	1stExon	10912478	0.38	1.17E-05	0.21	1.66E-02	0.30	6.53E-04	0.30
cg21453209	*[ZFAND1]*	8	--	82635831	0.32	3.47E-04	0.25	5.99E-03	0.33	2.23E-04	0.30
cg20594607	*LOC338799*	12	TSS200	122241438	0.33	1.94E-04	0.24	7.13E-03	0.31	3.81E-04	0.29
cg26840590	*PHF1*	6	5'UTR	33379330	0.32	2.86E-04	0.22	1.23E-02	0.34	1.39E-04	0.29
cg04530860	*B9D2*	19	TSS200	41870213	0.38	1.69E-05	0.24	7.20E-03	0.26	3.45E-03	0.29
cg17962547	*GPR123*	10	Body	134918974	0.32	3.57E-04	0.25	5.39E-03	0.31	4.39E-04	0.29
cg11480019	*ADK*	10	--	75936982	0.30	8.66E-04	0.32	3.13E-04	0.25	4.48E-03	0.29
cg09387749	*HOXD3*	2	TSS200	177028680	0.31	5.34E-04	0.24	7.14E-03	0.32	3.50E-04	0.29
cg27516159	*CACNA2D4*	12	Body	1904847	0.28	1.81E-03	0.22	1.27E-02	0.35	6.03E-05	0.28

B. Hypomethylated											
cg10494397	*CC2D2A*	4	Body	15593854	-0.39	7.89E-06	-0.24	6.61E-03	-0.42	1.35E-06	-0.35
cg27496299	*DNAJB6*	7	Body	157171202	-0.38	1.56E-05	-0.28	1.84E-03	-0.39	6.90E-06	-0.35
cg27632471	*C20orf96*	20	Body	259123	-0.27	2.41E-03	-0.39	8.78E-06	-0.39	9.42E-06	-0.35
cg07870603	***RPTOR***	17	Body	78880144	-0.28	1.37E-03	-0.38	1.43E-05	-0.33	2.28E-04	-0.33
cg09596252	***RPTOR***	17	Body	78655493	-0.41	3.00E-06	-0.28	1.62E-03	-0.30	7.06E-04	-0.33
cg01786585	*TIAM2*	6	--	155315188	-0.37	2.00E-05	-0.35	8.70E-05	-0.26	3.59E-03	-0.33
cg23523534	*NLRP11*	19	5'UTR	56347927	-0.33	1.45E-04	-0.32	3.12E-04	-0.32	3.59E-04	-0.32
cg16560389	*[TCERG1L]*	10	--	133318201	-0.34	9.50E-05	-0.22	1.32E-02	-0.40	3.60E-06	-0.32
cg05157878	*GCNT2*	6	--	10494860	-0.29	1.05E-03	-0.38	1.57E-05	-0.29	1.30E-03	-0.32
cg21473728	*MYOM2*	8	Body	2031651	-0.36	4.62E-05	-0.20	2.57E-02	-0.39	7.42E-06	-0.32
cg21385432	*EP400*	12	Body	132512858	-0.31	4.33E-04	-0.24	6.55E-03	-0.39	7.04E-06	-0.32
cg19279265	*ACOX3*	4	Body	8388670	-0.27	2.83E-03	-0.41	1.73E-06	-0.26	3.66E-03	-0.31
cg03561071	*[FAT3]*	11	--	91844355	-0.44	4.28E-07	-0.22	1.35E-02	-0.28	1.99E-03	-0.31
cg22896075	*GJD3*	17	1stExon	38518413	-0.40	5.30E-06	-0.21	2.04E-02	-0.32	3.09E-04	-0.31
cg13521944	*C22orf9*	22	Body	45596948	-0.27	2.45E-03	-0.28	1.69E-03	-0.37	2.97E-05	-0.30
cg11650926	*[KIAA0947]*	5	--	5568539	-0.30	8.71E-04	-0.19	3.40E-02	-0.43	6.91E-07	-0.30
cg21076890	*COL4A2*	13	Body	110965662	-0.30	8.61E-04	-0.19	3.93E-02	-0.43	5.51E-07	-0.30
cg03500617	*[FA2H]*	16	--	74812570	-0.31	4.27E-04	-0.19	3.11E-02	-0.41	2.86E-06	-0.30
cg02866700	*CARS2*	13	Body	111333333	-0.30	7.85E-04	-0.26	3.98E-03	-0.35	5.79E-05	-0.30
cg13920529	*SUGT1P1*	9	Body	33402426	-0.34	9.08E-05	-0.21	1.85E-02	-0.35	6.28E-05	-0.30

N.B. Gene names in brackets indicate the most proximal genes to the CpG probe based on Genome Studio. Genes with multiple significant probes are highlighted in blue.

Figure 1.

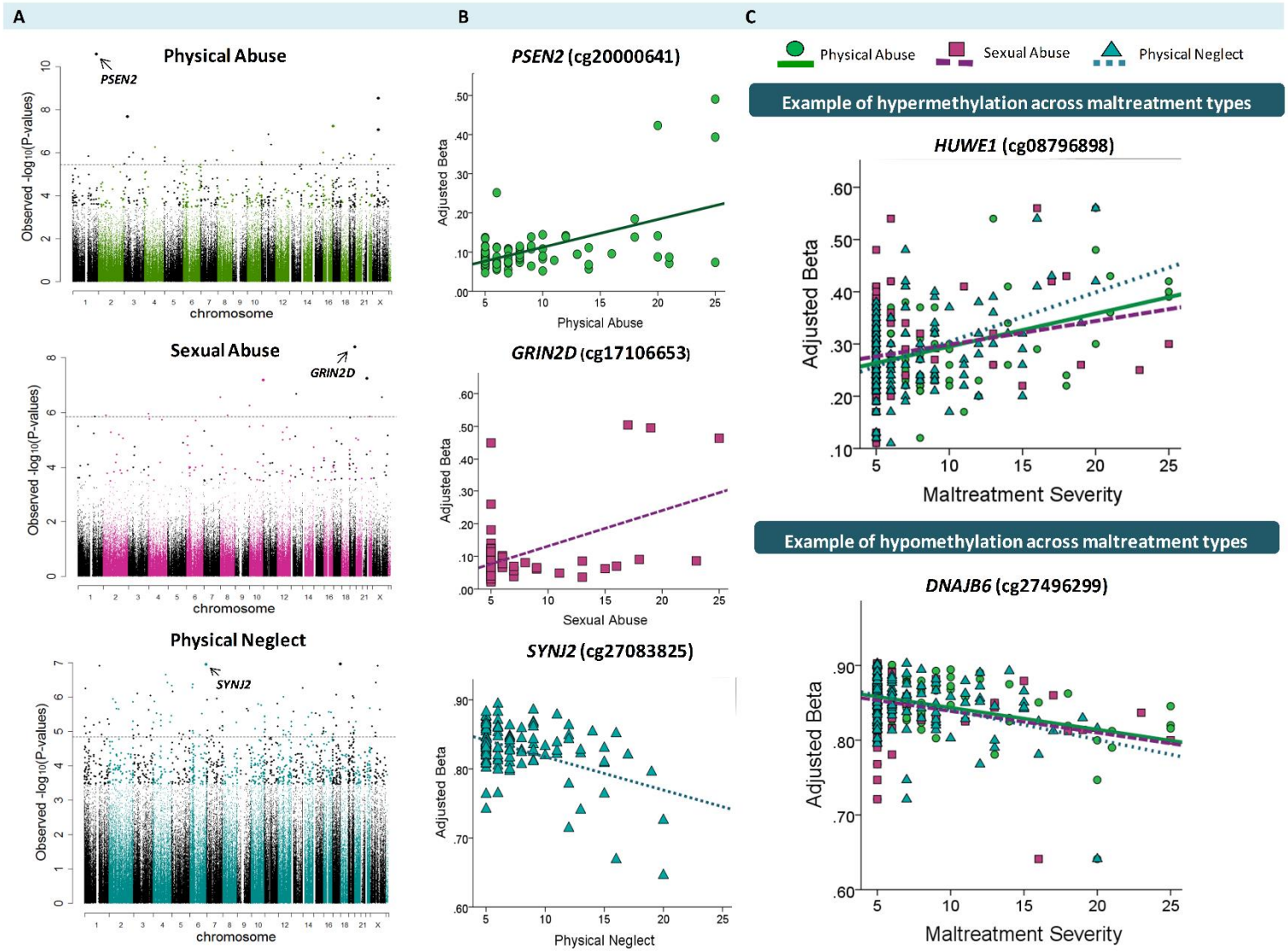
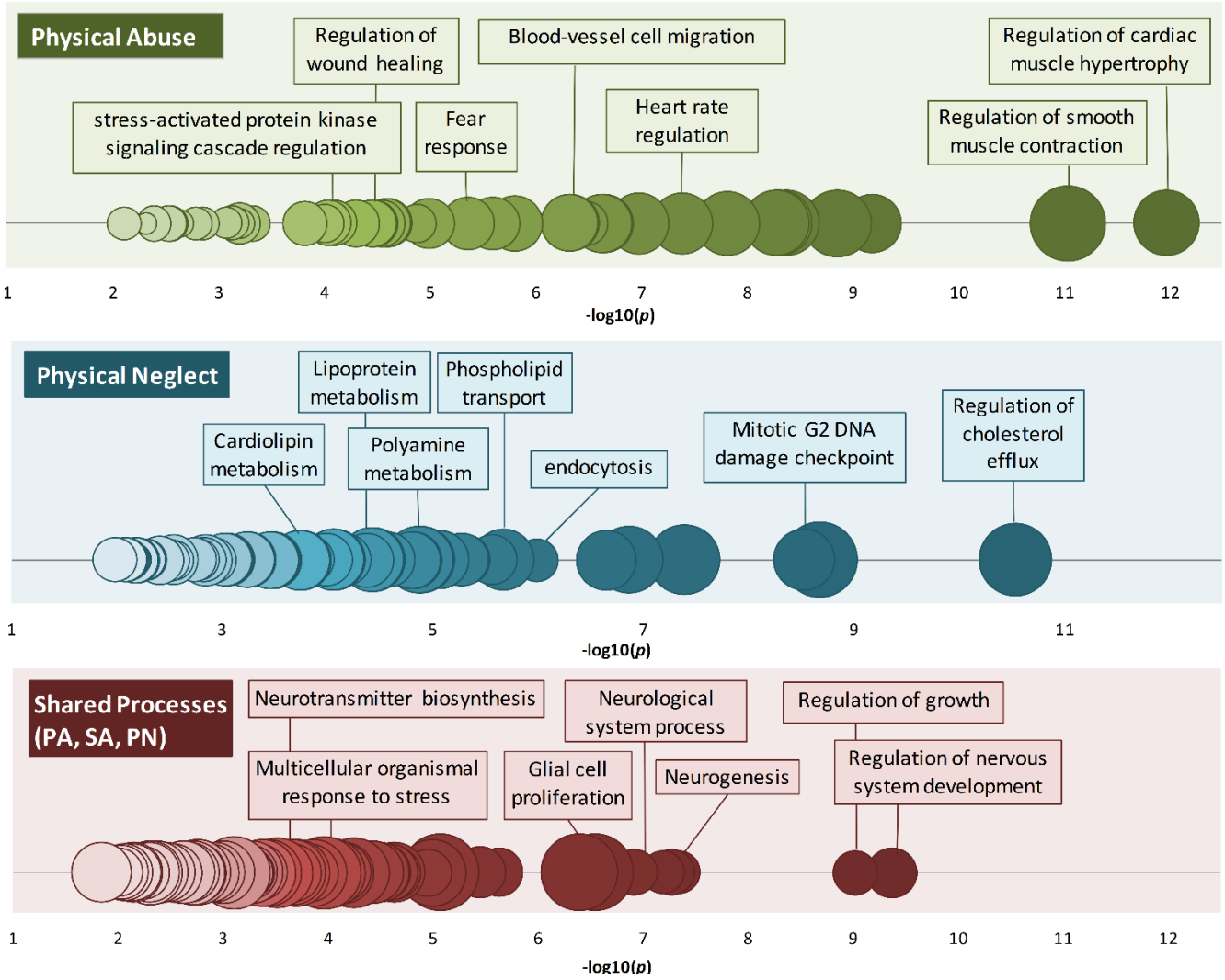

A
Physical Abuse
PSEN2
Observed -log10(P-values)
chromosome
Sexual Abuse
GRIN2D
Physical Neglect
SYNJ2
B
PSEN2 (cg20000641)
Adjusted Beta
Physical Abuse
GRIN2D (cg17106653)
Sexual Abuse
SYNJ2 (cg27083825)
Physical Neglect
C
Physical Abuse
Sexual Abuse
Physical Neglect
Example of hypermethylation across maltreatment types
HUWE1 (cg08796898)
Maltreatment Severity
Example of hypomethylation across maltreatment types
DNAJB6 (cg27496299)

Figure 2.


Physical Abuse
Regulation of wound healing
Blood-vessel cell migration
Regulation of cardiac muscle hypertrophy
stress-activated protein kinase signaling cascade regulation
Fear response
Heart rate regulation
Regulation of smooth muscle contraction
1 2 3 4 5 6 7 8 9 10 11 12
-log10(p)
Physical Neglect
Lipoprotein metabolism
Phospholipid transport
Cardiolipin metabolism
Polyamine metabolism
endocytosis
Mitotic G2 DNA damage checkpoint
Regulation of cholesterol efflux
1 3 5 7 9 11
-log10(p)
Shared Processes (PA, SA, PN)
Neurotransmitter biosynthesis
Neurological system process
Regulation of growth
Multicellular organismal response to stress
Glial cell proliferation
Neurogenesis
Regulation of nervous system development
1 2 3 4 5 6 7 8 9 10 11 12
-log10(p)